Amendments

Please enter the following amendments.

In the Specification

Replace the paragraph at page 8, lines 15-21 with the following amended paragraph:

This is demonstrated as follows:

Odd numbered template sequence:

"0": ATTTTTAT(CC) (SEQ ID NO.1)

"1": GTTTTTGT(CC) (SEQ ID NO.2)

Even numbered template sequence:

"0" : ACCCCCAC(TT) (SEQ ID NO.3)

"1": GCCCCCGC(TT) (SEQ ID NO.4)

Replace the paragraph at page 16, lines 14-30 with the following amended paragraph:

A target DNA fragment of 240 bp, containing internal sites for *Sfa*NI and *Eam*1104I was PCR-amplified from the bacteriophage Lambda genome (1507-1703) using primers containing restriction sites for *Eam*1104I and *Sfa*NI (the top fragment in Figure 2) respectively. In order to avoid cutting from the internal sites later, the PCR reaction was conducted with methylated deoxy-Cytosine nucleotides (m5-dCTP) instead of the usual deoxy-Cytosine (dCTP). PCR conditions: (50 µl): 10 mM KCl, 10mM $(NH_4)_2SO_4$, 20mM Tris-HCl, 0.1% Triton-X-100, pH 8.8 (Thermopol buffer, New England Biolabs), 2 mM Mg^{2+}, 200 µM dNTP (- dCTP) and 200 µM m5-dCTP (Amersham Pharmacia Biotech), 20 pmol Lambda primer #328 (5=-agactggcgatccctggcatcacccctccagcgtgttttat-3=; SEQ ID No [[1]] 5) and 20 mpol Lambda primer #329 (5=-gcactgataggcgtcactcttcgctgtacgctglccagatgt-3=; SEQ ID No [[2]] 6) (MWG biotech), 10 ng Lambda genome, 1U Vent polymerase (New England Biolabs). The PCR cycling was conducted with a PTC-200 (MJResearch). Hot start: 95EC, 5 minutes, 35 cycles consisting of: 95EC, 15 seconds, 58EC, 20 seconds, 72EC, 30 seconds. Complete extension step: 72EC, 5 minutes.

Replace the paragraph at page 17, lines 12-20 with the following amended paragraph:

The conversion step where the selection marker is attached (Figure 2, B) was carried out by ligating the fragment with the specific adapter and its associated signal sequence: 40 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP, pH 7.8 (Ligase buffer, Fermentas), 1.6 pmol adapter1 (5'=-Biotin-ggctaggtgctgatgaacgcatcg-3; SEQ ID No [[3']] 7; annealed to 5'=-tggacgatgcgttcatcagcacctagcc-3'; SEQ ID No [[4]] 8 (MWG-biotech), 1.9 pmol signal sequence 1 (1), 7.5 Weiss-U T4 DNA ligase (Fermentas). The incubation took place at room temperature (22EC) for 2.5 hours. The ligase was heat-inactivated at 65EC for 10 minutes.

Replace the paragraph at page 18, lines 7-21 with the following amended paragraph:

The selection step of the base conversion consists of selection and amplification of fragments which have had ligated to them both a signal sequence and a specific adapter (Figure 2,C). This was conducted using PCR under the following conditions (50 µl): 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, 0.1% Triton-X-100, pH 8.8 (Thermopol buffer, New England Biolabs), 2 mM Mg^{2+}, 200 µM dNTP (-dCTP) and 200 µM m5-dCTP (Amersham Pharmacia Biotech), 20 pmol adapter1-primer #332 (5'=-Biotin-ggctaggtgctgatgaacgcatcg-3'; SEQ ID No [[5]] 9) and 20 pmol Signal sequence 1-primer #340 (5'=-taatacgactcactatagcatgactcgagcctcttcgcga-3'; SEQ ID No [[5]] 10) (MWG-biotech), approx. 3.5 fmol ligated target DNA and 1U Vent polymerase (New England Biolabs). PCR cycling was conducted with a PTC-200 (MJResearch). Hot start: 95EC, 2 minutes, 20 cycles consisting of: 95EC, 15 seconds, 66EC, 20 seconds, 72EC, 30 seconds. Complete extension step: 72EC, 5 minutes. Well 2 in Figure 2 shows the results of the initial cycle of the sequencing method. The correct fragment of 380 bp was generated.

Replace the paragraph at page 18, line 23 through page 19, line 25 with the following amended paragraph:

In order to convert the next 4 bases, the PCR product was cleaned with the GibcoBRL purification system (Gibco) and then cut with *Sfa*NI and *Eam*1104I. It is possible for this to take place as the signal sequence and the adapter from the initial cycle contain a site for *Eam*1104I and *Sfa*NI respectively. As the sites were located in the primer region, they are not blocked by methylation during PCR. To increase the efficiency of the cutting reaction, the cuts were conducted serially under optimal cutting conditions. *Sfa*NI cutting: 100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$,1 mM DTT, pH 7.9 (NEB3, New England Biolabs), 1 µg methylated PCR fragment, 4U *Sfa*NI. Incubation at 37EC for 1 hour. Heat inactivation of the enzyme at 65EC for 10 minutes. A Micro Bio-Spin 6 column (BioRad) was used to clean the fragment. *Eam*1104I cutting: 33 mM Tris-acetate, 10 mM magnesium-acetate, 66 mM potassium-acetate, 0.1 mg/ml BSA, pH 7.9 (Tango Y^+, Fermentas), *Sfa*NI-digested PCR fragment, 10U *Eam*1104I. Incubated at 37EC for 1 hour, followed by heat inactivating at 65EC for 20 minutes. The digested product was cleaned by using the GibcoBRL purification system (Gibco). The initial step of the conversion was conducted corresponding to the initial cycle, except that a new signal sequence and its associated specific adapter (corresponding to the next 4 bases of the sequence) were added. To reduce potential carry-over problems, signal sequence 2 was designed with an different overhang from signal sequence 1. Ligation conditions: 40 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP, pH 7.8 (Ligase buffer, Fermentas), 0.5 pmol adapter2 (5'Biotin-cgacagtacgacggaccagcatcc-3'; SEQ ID No [[6]] 11 annealed to 5'-acgcggatgctggtccgtcgtactgtcg-3'; SEQ ID No [[7]] 12) (MWG-biotech), 1 pmol signal sequence 2 (2), 1 Weiss-U T4 DNA ligase (Fermentas). The incubation took place at room temperature (22EC) for 1.5 hours. The ligase was heat-inactivated at 65EC for 10 minutes. PCR amplification took place under the same conditions as the initial cycle, except that a new primer set (adapter2-primer #347, 5'-Biotin-cgacagtacgacggaccagcatcc-3; SEQ ID No [[6']] 11 and signal sequence 2-primer #343, 5'-taatacgactcactatagcatcgaatgaccgcctcttccact-3'; SEQ ID No [[8]] 13). Different primer sets for each sequencing cycle are preferable in order to minimise the danger of amplifying any remains from the previous sequencing cycle. The result of the PCR amplification in the second cycle is shown in Well 3 in Figure 4. The correct fragment of 523 bp was generated.

Replace the paragraph at page 19, line 27 through page 20, line 28 with the following amended paragraph:

Conversion of the next 4 bases and the PCR amplification followed the same pattern as described for Cycle 2: After a serial cutting with *Sfa*NI and *Eam*1104I, the fragment was ligated with signal sequence 3 (3) and adapter3 (5'-Biotin-atcgagcctggcatagcagcatca-3'; SEQ ID No [[9]] 14 annealed to 5'-aaactgatgctatgccaggctcgat-3; SEQ ID No [[10]] 15) (MWG-Biotech). The PCR amplification was conducted with the primer set: the adapter3-primer #353 (5'-atcgagcctggcatagcagcatca-3'; SEQ ID No [[9]] 14) and signal sequence 3-primer #345 (5'-taatacgactcactatagcaccgggcaggatagactcttcaggt-3'; SEQ ID No [[11]] 16). The result of the amplification in Cycle 3 is shown in Well 4 in Figure 4. Well 4 shows that two weak and one relatively strong bands are formed. The strong band comes closest to the expected length of 666 bp. The weak bands of the wrong size which can be seen in Well 4 (Cycle 3) and Well 3 (Cycle 2) are most probably the result of carry-over problems and mispriming. The weak band in Well 3 and the weak bands further down in Well 4 correspond to the size of the PCR fragment in the previous cycle. A possible explanation is that incomplete cutting of fragments in one cycle may function as a template in the next PCR cycle. Even though new primer sets in each cycle reduce this problem somewhat, the danger of mispriming is still present because of the enzyme sites the primers have in common. However, this type of mispriming may be eliminated by using more stringent annealing conditions during PCR (e.g. Mg^{2+}, temperature), the use of more discriminating polymerases, moving the *Eam*1104I site away from 3= on the sequence chain primer, choice of new sequences or immobilisation of the fragments (e.g. the biotin-streptavidin system on microbeads) before cutting so that those fragments entering the next cycle are guaranteed to have been cut. The best solution for other types of mispriming would be to choose new primer sequences and/or optimise PCR conditions. Mispriming events that are caused by the presence of superflous DNA material (e.g., excess adapters or non-ligated DNA) can be removed by using a strategy of thio-protecting the recessed strand of the adapter. Digestion with a 5'-3' exonuclease (e.g., T7 exonuclease or Lambda exonuclease) prior to PCR will only leave intact one strand of DNA ligated to the adapter. In embodiments where all target sequences are of equal lengths, size selection can be used to remove fragments of incorrect lengths during the process of biochemical conversion.

Replace the paragraph at page 21, lines 8-18 with the following amended paragraph:

A synthetic target DNA fragment of 66 bp, was generated by annealing the following oligonucleotides: # 003 (5'-PHO-GATCTTGGCTATTCGTCTCTTGGCTTTTCGTCTGATTGTAGACGCCAACGGGACATGATGATGAT-3'; SEQ ID No [[12]] 17) and # 004 (5'-[PHO-CATCATCATCATCATGTCCCGTTGGCGTCTACAATCAGACGAAAAGCCAAGAGACGAATAGCCAAG-3'; SEQ ID No [[13]] 18). As evident from the sequence the target molecule contains at the 3' end four consecutive ATG triplets. The annealed molecule corresponds to the nature of those fragments that are obtained after an initial preparation (described in Example 2) in that it has an overhang of 3 bases for signal sequence ligation and an overhang of 4 bases for ligation to a specific adapter.

Replace the paragraph at page 21, line 19 through page 22, line 12 with the following amended paragraph:

The conversion step where the selection marker is attached (Figure 5, B) was carried out by ligating 1 pmol of the fragment with the specific adapter and its associated signal sequence: 50 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 25 µg/ml BSA, pH 7.5 (ligase buffer, New England Biolabs), 1 pmol adapter # 005 (5'-GATGTAGATGCACTCCCGGACCTC-3'; SEQ ID No [[14]] 19 annealed to # 006 5'-GAGGTCCGGGAGTGCATCTA-3'; SEQ ID No [[15]] 20) (MWG-biotech), 1 pmol signal seqeunce # 001 (5'-TGTGTCCGCGTGGCTCTTCTGATCTTGGCTTTTCGTCTCTTGGCTATTCGTCT-3'; SEQ ID No [[16]] 21, annealed to # 002 5'-PHO-ATCAGACGAATAGCCAAGAGACGAAAAGCCAAGATCAGAAGAGCCACGCGGACACA-3'; SEQ ID No [[17]] 22) (MWG-biotech), 100 units T4 DNA ligase (New England Biolabs). The incubation took place at 22 1C in a PTC-200 thermal cycler (MJResearch) for 1 hour. Selection and amplification of those molecules being successfully ligated to both the adapter and the signal sequence adapter were conducted using PCR under the following conditions (50µl): 10 mM KCl, 10 mM $(NH_4)SO_4$, 20 mM Tris-HCl, 0.1% Triton-X-100, pH

8.8 (Thermopol buffer, New England Biolabs), 2 mM Mg^{2+}, 200 μM dNTPs (Amersham Bioscience), 10 pmol adapter primer # 006 (5'-GAGGTCCGGGAGTGCATCTA-3'; SEQ ID No [[18]] 23) (MWG-Biotech) and 10 pmol signal chain primer # 007 (5'-TGTGTCCGCGTGGCTCTTCT-3'; SEQ ID No [[19]] 24) (MWG-Biotech), approx. 1 pmol ligated target DNA and 0.2 U Vent polymerase (New England Biolabs). PCR cycling was conducted with a PTC-200 (MJReasearch). Hot start: 951C, 2 minutes, 25 cycles consisting of: 951C, 15 seconds, 59.31C, 20 seconds, 721C, 20 seconds. Complete extension step: 721C, 60 seconds. Well 2 in Figure 6 shows the results of the initial cycle of the sequencing method. The correct fragment of 142 bp was generated.

In the Sequence Listing

Please replace the sequence listing currently of record with the Substitute Sequence listing (pages 1-6) submitted herewith.